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I 49.28/3:62

BACTERIAL GILL DISEASE OF FRESHWATER FISHES[1/]

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FISH DISEASE LEAFLET 62

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Division of Fishery Research
Washington, D. C. 20240

1981

[1/] Revision of *Fish Disease Leaflet* 19, same title and author, 1970. Reprinted August 1984

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INTRODUCTION

Bacterial gill disease (BGD) was first described by Davis (1926, 1927), who observed it in fry and fingerling brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) in hatcheries in Vermont. The affected trout were kept in dirt-bottom ponds and were not crowded; daily mortalities were very low. Mortalities rapidly increased when the water temperature increased and diminished when the temperature decreased. Examination of the diseased trout revealed clubbing of gill filaments. In microscopic examination of wet mounts of the filaments, Davis found that the gill surface was covered with closely adhering strands of long, thin bacteria. He called the condition bacterial gill disease, but did not attempt to isolate or identify the bacteria.

Detailed descriptions of BGD were published by Davis (1953), Wood and Yasutake (1957), Ghittino (1967), Bullock (1972), Wood (1974), Kimura et al. (1978), and Wakabayashi et al. (1980). On the basis of these descriptions, it seems obvious that bacterial gill disease is a complex disease in which more than one type of bacterium may be involved. Environmental conditions play an extremely important role in the occurrence and severity of the disease; losses sometimes jump from nil to 50% within 1 day.

Pathologic changes in gills are caused also by other diseases, among which six are particularly important: (1) nutritional gill disease, caused by deficiency of pantothenic acid; (2) aneurysmal, or hemorrhagic gill disease, caused by various toxic agents or blocking of gill capillaries by the parasites (Wood and Yasutake 1957); (3) necrotic gill disease of carp, caused by pollution of pond water with ammonia and other products of fish metabolism (Schreckenbach et al. 1975); (5) mycotic gill necrosis, caused by *Branchiomyces* (Meyer and Robinson 1973; Neish and Hughes 1980); and (6) columnaris disease (Davis 1953).

Gills of fishes serve as a respiratory and excretory organ. Basically they consist of a large network of capillaries in which blood is separated from water by only one or two layers of cells. The presence of films of bacteria, proliferation of tissue, and loss of surface by clubbing and fusing results in impaired respiration, hindering of excretion of nitrogeneous waste materials, and disturbance of osmotic balance. These changes have a detrimental effect on the health of fish and threaten their survival. Therefore the prevention and treatment of bacterial and other gill diseases are important factors in fish culture.

ETIOLOGY

The study of the etiology of BGD has a long and complicated history. In early work, neither Davis (1926, 1927) nor Borg (1960) obtained pure cultures of the filamentous bacteria present in large numbers on the surface of gills of salmonids suffering from gill disease. Rucker et al. (1949, 1952) obtained pure cultures of the filamentous bacteria and considered them as belonging to the genus *Cytophaga*. However, they could not reproduce the disease with pure cultures.

Bullock (1972), in monographic studies, isolated bacteria from columnaris disease and BGD and assigned them to the myxobacteria. In the 8th edition of Bergey's Manual, bacteria causing columnaris disease were assigned to the group of gliding bacteria. This group includes, among others, the family *Cytophagaceae* and the genera *Cytophaga* and *Flexibacter* (*F. columnaris*). Therefore the bacteria studied by Bullock (1972) should now be assigned to these two genera, with gill disease bacteria belonging to the genus *Cytophaga*. Bullock's efforts to experimentally reproduce BGD with strains of *Cytophaga* were only partly successful. Adding suspensions of pure *Cytophaga* cultures to water in which fish were held failed to produce gill disease. However, the disease could be regularly transmitted to healthy, but stressed, trout by introducing trout with advanced gill disease.

The most recent studies on etiology, carried out in Japan and the northwestern United States by Kimura et al. (1978), Wakabayashi (1980), and Wakabayashi et al. (1980), resulted in isolation of gram-negative, filamentous bacteria, producing nonsoluble yellow pigment, and small circular nonspreading colonies. The cells of these bacteria did not show flexing or gliding movements. On the basis of these characteristics, they were classified as *Flavobacterium*. However, Weeks (1974), who is the author of the section on *Flavobacterium* in Bergey's Manual, indicated that members of this genus form a heterogenous group. He also stated that "differentiation of nonmotile flavobacteria from *Cytophaga* is a major unresolved problem." According to Wakabayashi et al. (1980), gill disease could be reproduced experimentally by cultures isolated in Japan and the United States. These flavobacteria form a uniform group, though there are regional serological differences between the Japanese and American cultures. Mortalities in the inoculated fish were much lower than in spontaneous outbreaks of BGD. These results bring much closer the explanation of the etiology of BGD. Nevertheless, as stated by Weeks (1974), the problem of establishing the taxonomic position of nonmotile flavobacteria from *Cytophaga* needs to be resolved. This is particularly important because cultures of bacteria isolated from fish with BGD and studied by Bullock (1972) produced spreading colonies characteristic for *Cytophaga*.

The relationship between flavobacteria and cytophagas was the subject of a 1980 international symposium. Dr. H. Reichenbach, organizer, made the following remarks (personal communication): "The question whether cytophagas or flavobacteria are the causative agent of gill disease is difficult to decide at the moment. I am convinced that there are close connections between the cytophagas and certain groups of flavobacteria, so that the question may be improperly put. Perhaps said flavobacteria are rather non-gliding, or conditionally gliding cytophagas? The symposium has not provided the final answer, but a number of interesting observations about chemical correspondences between the two groups of organisms."

Another type of filamentous gram-negative bacterium was described from a gill disease in salmonid hatcheries in British Columbia (Hoskins 1976).

These bacteria were described as "fusobacteria" due to their spindle shape and granular staining. All attempts to culture these bacteria failed. Therefore they have not been sufficiently described to permit positive identification.

An interesting problem in the etiology of BGD is that of determining the relative importance of bacteria described by Borg (1960), Rucker et al. (1949, 1952), Bullock (1972), and Wood (1974), which are believed to be *Cytophaga*, and those described from Japan (Kimura et al. 1978; Wakabayashi et al. 1980) and the northwestern United States as flavobacteria. Bacterial gill disease was considered by various early authors to be of a nonuniform etiology. It will be interesting to find out whether there is any relationship between the various types of bacteria isolated from different outbreaks of this disease.

It is now generally accepted by epidemiologists that the balance between potential pathogens and their host is the normal state. This relationship results in commensal coexistence and the absence of overt disease. This balance can be upset by introduction of an exotic pathogen or a new host to the environment, or by changes in the nature of the pathogen, host, or the environment. Changes in the pathogen may be caused by the appearance of different plasmids, the presence of capsules, or any other genetic rearrangements. Changes of host susceptibility may be immunologic or caused by age. Environmental changes in factors such as temperature, pH, general chemistry of water, availability of dissolved oxygen, crowding, or pollution are very important.

Bacterial gill disease seems to be an excellent indicator of these multiple factors. Under well-balanced conditions, microorganisms associated with it are usually present on the fish or in the environment, but there is no overt disease. The overt disease and its severity depend on the degree to which the balance is upset. When conditions become "normal" again the disease is likely to disappear without treatment.

CLINICAL SIGNS AND PATHOLOGY

One of the first signs of bacterial gill disease is loss of appetite and the orientation of affected fishes toward the flow of water. If undisturbed, the fish seem to position themselves equidistant from each other, probably to make optimum use of the available space and dissolved oxygen. Examination of gills in a wet mount under a high dry objective of the microscope reveals from slight to advanced clubbing of gill filaments, resulting in eventual fusing of lamellae and sometimes even of the filaments.

The arrangement of bacteria on the surface of gills is variable. Numerous published illustrations of bacterial gill disease, made by transmitted light or electron microscopy or by observation in vivo, show the bacteria as layers or strands adhering closely to the gills. In other

illustrations bacteria are protruding away from the surface of the gills, are actively oscillating, and are arranged in clumps somewhat like those in columnaris disease.

In an outbreak of typical BGD there is proliferation of gill epithelium but never necrosis. Necrosis is characteristic of columnaris disease of the gills. Bacteria involved in both diseases have a similar filamentous shape.

The general pathology of BGD is most likely caused by restrictions on the respiratory and excretory functions of the gills. Therefore, in acute outbreaks, heavy losses may take place within 1 or 2 days after the onset. Acute outbreaks usually occur among juvenile salmonids from the swim-up stage to yearlings up to 5 cm long. Acute outbreaks of bacterial gill disease are less likely to occur in larger and older fish. The severity of the disease depends on the quality of environmental conditions.

DIAGNOSIS

Diagnosis of bacterial gill disease is based chiefly on microscopic examination of the gills for the presence of thin, long filamentous bacteria, proliferation of gill epithelium, and clubbing and fusing of the gill filaments. If bacteria adhere closely to the epithelium, or are not abundant, and their presence cannot be positively established in wet mounts, excised gill lamellae should be scraped with a scalpel or mashed between the slides, and the preparations fixed and stained--preferably gram-stained. Using this technique, one can easily see even a few bacteria as long, gram-negative filaments. Isolation of a pure culture of gill disease bacteria is not necessary for routine diagnosis.

SOURCE AND RESERVOIR OF INFECTION

Nothing is known about the existence and survival of the agents of bacterial gill disease outside the fish. Davis (1926, 1927) reported that if fingerling trout of the same lots were divided and some were maintained in spring water and others in open stream water, only those in stream water developed bacterial gill disease. This relation indicates that streams may harbor older fish that serve as a source of bacteria. Also, trout in water received from a pond with diseased trout developed gill disease. Bullock (1972) showed that gill disease can be reproduced in experimental trout by introducing trout with gill disease. This evidence indicates that carrier fish are likely to be the source of specific gill disease bacteria. He also found that older salmonids contained antibodies against isolates of bacteria from BGD, indicating that exposure of hatchery fish to bacterial gill disease is a common occurrence.

MODE OF TRANSMISSION

Bacterial gill disease is transmitted by contact with diseased fishes or by contaminated water.

ENVIRONMENT AND BACTERIAL GILL DISEASE

It is a well-documented observation that outbreaks of communicable diseases are influenced by the environment, and this influence is particularly clear in bacterial gill disease. Burrows (1964) showed that the accumulation of ammonia, in its un-ionized form, originating as an excretory product of fish, is conducive to outbreaks of this disease. This observation was confirmed by Larmoyeux and Piper (1973), who found that pathological changes in gills and the presence of gill disease bacteria were to be expected in fish kept in water reused several times. Smith and Piper (1975) showed that rainbow trout suffered advanced pathology of gills, as well as focal necrosis in livers, when kept in tanks where water was reused six times and contained 1.5 ppm ammonia. Brauhn et al. (1976) showed that the growth and health of rainbow trout were impaired when dissolved oxygen concentration was 5.0 ppm, or less, and ammonia reached 0.5 ppm.

Walters and Plumb (1980) showed that environmental stress contributed to increased colonization of fish by bacteria and to systemic bacteremia. They stressed channel catfish (*Ictalurus punctatus*) by reducing dissolved oxygen to 1.5 mg/L and increasing total dissolved ammonia to 1.2 mg/L. *Aeromonas hydrophila* was isolated from about seven times more stressed than control fish, and 43% of the stressed fish also suffered from *Edwardsiella tarda*. Only 7% of the control fish were detectable normal carriers of *E. tarda*. It is, therefore, not surprising that crowding of fishes, resulting in excessive accumulation of products of fish metabolism and lowering of dissolved oxygen, is conducive to proliferation of filamentous bacteria associated with BGD.

INCUBATION AND COMMUNICABILITY

In BGD, as in other fish diseases, the period of incubation depends on the age of the fish, environmental conditions, and virulence of the bacteria. Davis (1926, 1927) reported that BGD occurred even at the swim-up stage, or soon afterward. In such cases the period of incubation could not be longer than a few days.

In cases described by Burrows (1964), Bullock (1972), and Larmoyeux and Piper (1973) the incubation period was probably several weeks, or months, in tanks containing a borderline concentration of toxic products of fish metabolism. In contrast, in hatcheries with juvenile salmonids, usually only 1 or 2 days elapse between cessation of feeding and the appearance of numerous gill disease bacteria on the gill epithelium. Thus the incubation time varies greatly with environmental conditions.

Gill disease in juvenile salmonids is most likely to occur when the water supply contains older salmonids or other fishes that are carriers.

SUSCEPTIBILITY AND RESISTANCE

All juvenile salmonids and most freshwater fishes are probably susceptible to bacterial gill disease under environmental conditions favorable to the bacterium and stressful to the fish.

GEOGRAPHIC RANGE

At first bacterial gill disease was described only in North America. More recent reports indicate that its distribution is worldwide.

CONTROL OF BACTERIAL GILL DISEASE

Immunity

Davis (1926, 1927) observed that outbreaks of bacterial gill disease seldom reoccur among salmonids successfully treated for gill disease by dipping them in a solution of copper sulfate. He assumed that this nonrecurrence was the result of acquired immunity. Bullock (1972) confirmed this assumption by showing that in fish hatcheries most of the salmonids 1 year old or older had serum antibodies against the filamentous bacteria that he believed to be the cause of BGD. No research has yet been reported on purposeful immunization of fish against BGD.

Sanitation

Proper sanitation is probably the most effective protection against bacterial gill disease. Davis (1926, 1927) reported that BGD did not occur in hatcheries supplied with water from fish-free sources.

Outbreaks of BGD were observed to be associated with environmental stress by Borg (1960), Burrows (1964), Bullock (1972), Larmoyeux and Piper (1973), and others. Experimental transmission of BGD was difficult or impossible unless the test fishes were exposed to stress. Presence of carrier fish in the water supply is obviously undesirable.

Therapy[1/]

Therapy of bacterial gill disease is well developed and effective. Although the etiology of BGD is still not completely explained, one fact

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1/ The use of drugs and chemicals in the United States must be in accordance with current Federal and State laws and regulations. Mention of product name does not imply endorsement by the U.S. Fish and Wildlife Service.

seems to be well established: when bacteria on the gills are removed by treatment, fish recover rapidly. According to Davis (1926, 1927) subsequent treatments are seldom necessary because trout that recover become immune to BGD.

Numerous chemical disinfectants are effective. Organic mecurials are effective but have a narrow margin of safety. They are no longer used in the United States because they pollute the water and are carcinogenic; laws and regulations sharply restrict or ban their use.

The most widely used disinfectants are quaternary ammonia compounds, such as benzalkonium chlorides, available as Hyamine 1622 (98.8% active ingredient) and Hyamine 3500 (50% active ingredient). Another compound of this type is Roccal, available in several concentrations. Benzalkonium chlorides are used in concentrations of 1 to 2 ppm (calculated on the basis of the active ingredient) as a prolonged bath for up to 1 h. Caution is necessary because the margin of safety is narrow, particularly in soft water.

Another chemical used is 6,7 dihydrodipirido [1,2a: 2',1'-c]pyrazinedium dibromide, available commercially as Diquat. Diquat is recommended at a concentration of 8.4 to 16.8 ppm of the formulated material, or 2 to 4 ppm on the basis of active ingredient (Diquat cation).

Wood (1974) also recommended the use of potassium permanganate ($KMnO_4$), a chemical of wide application in fish culture. It is recommended as a 1-h bath at a concentration of 1 to 2 ppm. Wood (1974) listed certain precautions to be taken in using $KMnO_4$.

Copper sulfate, $CuSO_4 \cdot 5H_2O$, was recommended by Davis (1926) at a concentration of 1:2,000 for a 1-min dip. Although it is effective, dipping is now seldom used. Copper sulfate may be effective as a prolonged bath for 1 h at a concentration of 1 ppm. It should be tested first with only a few diseased fish, to avoid unforeseeable acute toxic effects. Chloramine-T has recently been used for the treatment of BGD by Europeans (From 1980) with good results. However, it is unlikely that the use of this compound will be allowed in the United States because of its suspected carcinogenicity.

CONCLUSION

Bacterial gill disease is a disease of fishes in which filamentous bacteria, of still uncertain taxonomic position, infect the gill surface. Frank and acute BGD, with losses of fish, is usually triggered by unfavorable environmental conditions. Treatment with certain chemicals is usually effective, particularly if accompanied by improvement of the environmental conditions.

ACKNOWLEDGMENT

I sincerely appreciate the assistance of G. L. Bullock, who reviewed the manuscript and made suggestions.

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